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## GATCGGenerator: New Software for Generation of Quasirandom Nucleotide Sequences

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### Abstract

**Introduction.** In recent decades, knowledge about DNA has been increasingly used to solve biological problems (calculations using DNA, long-term storage of information). Principally, we are talking about cases when it is required to select artificial nucleotide sequences. Special programs are used to create them. However, existing generators do not take into account the physicochemical properties of DNA and do not allow obtaining sequences with a pronounced “non-biological” structure. In fact, they generate sequences by distributing nucleotides randomly. The objective of this work is to create a generator of quasirandom sequences with a special nucleotide structure. It should take into account some physicochemical features of nucleotide structures, and it will be involved in storing non-biological information in DNA.

**Materials and Methods.** A new GATCGGenerator software for generating quasirandom sequences of nucleotides was described. It was presented as SaaS (from “software as a service”), which provided its availability from various devices and platforms. The program generated sequences of a certain structure taking into account the guanine-cytosine (GC) composition and the content of dinucleotides. The performance of the new program algorithm was presented. The requirements for the generated nucleotide sequences were set using a chat in Telegram, the interaction with the user was clearly shown. The differences between the input parameters and the specific nucleotide structures obtained as a result of the program were determined and generalized. Also, the time costs of generating sequences for different input data were given in comparison. Short sequences differing in type, length, GC composition and dinucleotide content were studied. The tabular form shows how the input and output parameters are correlated in this case.

**Results.** The developed software was compared to existing nucleotide sequence generators. It has been established that the generated sequences differ in structure from the known DNA sequences of living organisms, which means that they can be used as auxiliary or masking oligonucleotides suitable for molecular biological manipulations (e.g., amplification reactions), as well as for storing non-biological information (images, texts, etc.) in DNA molecules. The proposed solution makes it possible to form specific sequences from 20 to 5,000 nucleotides long with a given number of dinucleotides and without homopolymer fragments. More stringent generation conditions remove known limitations and provide the creation of quasirandom sequences of nucleotides according to specified input parameters. In addition to the number and length of sequences, it is possible to determine the GC composition, the content of dinucleotides, and the nature of the nucleic acid (DNA or RNA) in advance. Examples of short sequences differing in length, GC composition and dinucleotide content are given. The obtained 30-nucleotide sequences were tested. The absence of 100 % homology with known DNA sequences of living organisms was established. The maximum coincidence was observed for the generated sequences with a length of 25 nucleotides (similarity of about 80 %). Thus, it has been proved that GATCGGenerator can generate non-biological nucleotide sequences with high efficiency.

**Discussion and Conclusion.** The new generator provides the creation of nucleotide sequences *in silico* with a given GC composition. The solution makes it possible to exclude homopolymer fragments, which improves qualitatively the physicochemical stability of sequences.

**Keywords:** GATCGGenerator, nucleotide sequences generator, synthetic nucleic acids, random sequences, data storage in DNA, steganography, NYRN-oligonucleotides, calculations with DNA, cryptography, DNA-tagging in hydrology

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Научная статья

## GATCGGenerator: новый генератор для создания квазислучайных нуклеотидных последовательностей

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### Аннотация

**Введение.** В последние десятилетия знания о ДНК все шире применяются для решения небологических задач (вычисления с помощью ДНК, долговременное хранение информации). В первую очередь речь идет о случаях, когда необходимо подобрать искусственные нуклеотидные последовательности. Для их создания используются специальные программы. Однако существующие генераторы не учитывают физико-химические свойства ДНК и не позволяют получать последовательности с явно выраженной «небиологической» структурой. Фактически они генерируют последовательности, распределяя нуклеотиды случайным образом. Целью данной работы является создание генератора квазислучайных последовательностей с особой нуклеотидной структурой. Он должен учитывать некоторые физико-химические особенности нуклеотидных структур и будет задействован при хранении небологической информации в ДНК.

**Материалы и методы.** Описано новое программное обеспечение GATCGGenerator для генерации квазислучайных последовательностей нуклеотидов. Оно предоставляется как SaaS (от англ. software as a service — программное обеспечение как услуга), что обеспечивает его доступность с разных устройств и платформ. Программа генерирует последовательности определенной структуры с учетом гуанин-цитозинового (GC) состава и содержания динуклеотидов. Представлена работа алгоритма новой программы. Требования к сгенерированным нуклеотидным последовательностям заданы с помощью чата в «Телеграм» (Telegram), наглядно показано взаимодействие с пользователем. Определены и обобщены различия входных параметров и получаемых в результате работы программы конкретных нуклеотидных структур. Также в сопоставлении даны временные затраты генерации последовательностей при различных входных данных. Изучены короткие последовательности, различающиеся по типу, длине, GC-составу и содержанию динуклеотидов. В табличном виде показано, как в этом случае соотносятся входные и выходные параметры.

**Результаты исследования.** Созданное программное обеспечение сравнили с существующими генераторами нуклеотидных последовательностей. Установлено, что генерируемые последовательности отличаются по структуре от известных ДНК-последовательностей живых организмов, а значит, могут быть использованы в качестве вспомогательных или маскирующих олигонуклеотидов, пригодных для молекулярно-биологических

манипуляций (например — реакции амплификации), а также для хранения в молекулах ДНК небологической информации (изображений, текстов и т. д.). Предложенное решение дает возможность формировать специфические последовательности длиной от 20 до 5 000 нуклеотидов с заданным числом динуклеотидов и без гомополимерных участков. Более жесткие условия генерации снимают известные ограничения и позволяют создавать квазислучайные последовательности нуклеотидов по заданным входным параметрам. Кроме количества и длины последовательностей можно заранее определить GC-состав, содержание динуклеотидов и природу нуклеиновой кислоты (ДНК или РНК). Приводятся примеры коротких последовательностей, различающихся по длине, GC-составу и содержанию динуклеотидов. Полученные 30-нуклеотидные последовательности прошли проверку. Установлено отсутствие 100-процентной гомологии с известными ДНК-последовательностями живых организмов. Максимальное совпадение наблюдалось для сгенерированных последовательностей длиной 25 нуклеотидов (сходство около 80 %). Таким образом доказано, что GATCGGenerator может с высокой эффективностью генерировать небологические нуклеотидные последовательности.

**Обсуждение и заключение.** Новый генератор позволяет создавать нуклеотидные последовательности *in silico* с заданным GC-составом. Решение дает возможность исключить гомополимерные фрагменты, что качественно улучшает физико-химическую стабильность последовательностей.

**Ключевые слова:** GATCGGenerator, генератор нуклеотидных последовательностей, синтетические нуклеиновые кислоты, случайные последовательности, хранение данных в ДНК, стеганография, NYRN-олигонуклеотиды, вычисления с помощью ДНК, криптография, ДНК-метчики в гидрологии

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**Introduction.** DNA is a unique biopolymer that provides storage, transmission and reproduction of genetic information in living organisms. DNA molecules consist of four types of nucleotides containing nitrogenous bases: adenine (A), guanine (G), cytosine (C), thymine (T). Their possible combinations provide nucleotide sequences forming functional genetic elements. In molecular biology and genetics, the basic investigations are carried out on nucleotide sequences of living organisms, but there is an increasing need to create artificial sequences, especially, when solving non-biological tasks (e.g., DNA calculations [1, 2], storage in DNA [3], cryptography [4], DNA tags in hydrology [5], etc.).

It is expected that by the end of 2040, the volume of information will reach several yottabytes ( $10^{24}$ ), which requires its structuring and storage. Both of these processes affect significantly the consumption of energy resources, as well as the production of storage devices and peripheral devices (hard drives, solid-state drives). To store such an amount of information, more than  $10^9$  kg of extra pure silicon is required [6], which may not be enough. The solution is seen in using the principles of DNA to work with large-scale amounts of data.

Nucleotide sequences are easily digitized by assigning the corresponding binary codes to individual nucleotides [7–11] or blocks of nucleotides [12–14]; therefore, text, graphic or multimedia files can be converted into nucleotide sequences [15–18]. Artificial nucleotide sequences can be made manually or generated using special software (DNA generators), depending on the tasks being solved. Some DNA generators were developed as independent applications,

others — as part of software packages designed to solve general [19]<sup>1, 2, 3, 4, 5</sup> or specific tasks [20]. As a rule, DNA generators are developed on the basis of combinatorial approaches and produce random sequences of a given length of guanine-cytosine (GC) composition. However, such software solutions do not take into account the chemical properties of nucleotides and do not provide obtaining sequences with a certain structure (e.g., without homopolymer sites or long repeating motifs). Therefore, the sequences created by such generators cannot always be reproduced in the laboratory. Moreover, such sequences may be identical to DNA fragments existing in nature, which introduces ambiguity when trying to encode information of a non-biological nature.

The presented work is aimed at creating a generator of nucleotide sequences of a special structure that can be used when encoding text, graphic and other information in DNA molecules.

**Materials and Methods.** The criteria that should be kept in mind when creating sequences were defined. The need to vary the GC composition, set a certain number of dinucleotides, and exclude homopolymer sites in sequences was taken into account.

A team of authors has developed the GATCGGenerator program in Python 3.6 (Anaconda distribution)<sup>6</sup>. To create a bot<sup>7</sup> in Telegram, Numpy 1.19 [21] and the Python GATCGGenerator library were used. The solution was provided as SaaS (from “software as a service”), which opened up the possibility of access from different devices and platforms.

Input parameters included the number of sequences, their length, GC composition, and dinucleotide content. The generator excluded repeats with a length of two nucleotides more than four times. The result was presented as a CSV file, which contained the following information: sequence, GC composition, and the number of all nucleotides.

Repeats and homopolymer fragments were stored as a separate list. First, a sequence of four elements was randomly generated (`random.choice(nuc)`, where `nuc = 'ACGT'`). Then the search for repetitions was performed. If there was at least one item from the list, a new random generation was performed. Next, the GC and NN composition was calculated. If the NN composition did not match the user-defined range, the paired nucleotide was replaced randomly and the GC composition was recalculated. If the sequence matched the input parameters, it was written to a set of sequences.

Below is the operation of the program algorithm.

Type, GCmin, GCmax — range of possible GC content, NNmin, NNmax — range of possible dinucleotide content  
NN%, N — quantity, S — sequence, l — sequence length, count — total number of sequences

Pseudocode

Start

Input (Type, GC, NN, N)

Comprehension of a list of repeating motifs, homopolymer sites `rep.list`

Count = 0

sequences = set()

IF  $i \leq N$ ?

IF (`rep.list(k)  $\subset$  S`)?

Return to **step 1**.

ELSE

`NN = len(DI_REGEX.findall(".join(S)))`

`NN_perc = (NN  $\times$  2 / l)  $\times$  100`

IF `NNmin  $\leq$  NN_perc  $\leq$  NNmax`

`GC = S.count('G') + S.count('C') / l  $\times$  100`

IF `GCmin  $\leq$  GC  $\leq$  GCmax`

IF `type == DNA`

**Step 2.**

`A_perc = S.count('A') / l  $\times$  100`

<sup>1</sup> Nucleotide Sequence Generator. nucleotide-generator.herokuapp.com. URL: <https://nucleotide-generator.herokuapp.com/> (accessed: 01.12.2022).

<sup>2</sup> DNA Sequence Tools: Random Sequence Generator. molbiotools.com. URL: <http://www.molbiotools.com/randomsequencegenerator.html> (accessed: 01.12.2022).

<sup>3</sup> Random DNA Sequence Generator. faculty.ucr.edu. URL: <http://www.faculty.ucr.edu/~mmaduro/random.htm> (accessed: 02.12.2022).

<sup>4</sup> Random DNA Sequence GenScript. genscript.com. URL: [https://www.genscript.com/sms2/random\\_dna.html](https://www.genscript.com/sms2/random_dna.html) (accessed: 04.12.2022).

<sup>5</sup> Random DNA Generator. Computer software. URL: <http://54.235.254.95/cgi-bin/gd/gdRandDNA.cgi> (accessed: 04.12.2022).

<sup>6</sup> Anaconda. Anaconda Inc. anaconda.com. URL: <https://www.anaconda.com/> (accessed: 20.01.2023).

<sup>7</sup> Python telegram bot. github.com. URL: <https://github.com/python-telegram-bot/python-telegram-bot> (accessed: 01.12.2022).

```

G_perc = S.count('G') / 1 × 100
C_perc = S.count('C') / 1 × 100
T_perc = S.count('T') / 1 × 100
U_perc = S.count('U') / 1 × 100
Count = count + 1
sequences.add(S)
ELSE S = S.replace('T', 'U')
Step 2.)
ELSE
    Return to step 1.
ELSE
    Random replacement of the second repeated character,
    GC = S.count('G') + S.count('C') / 1 × 100
Output Sequences: (S, GC%, NN%, A%, G%, C%, T/U%)
End

```

The requirements for the generated nucleotide sequences were set using Telegram chat. An example of user interaction is shown in Figure 1.

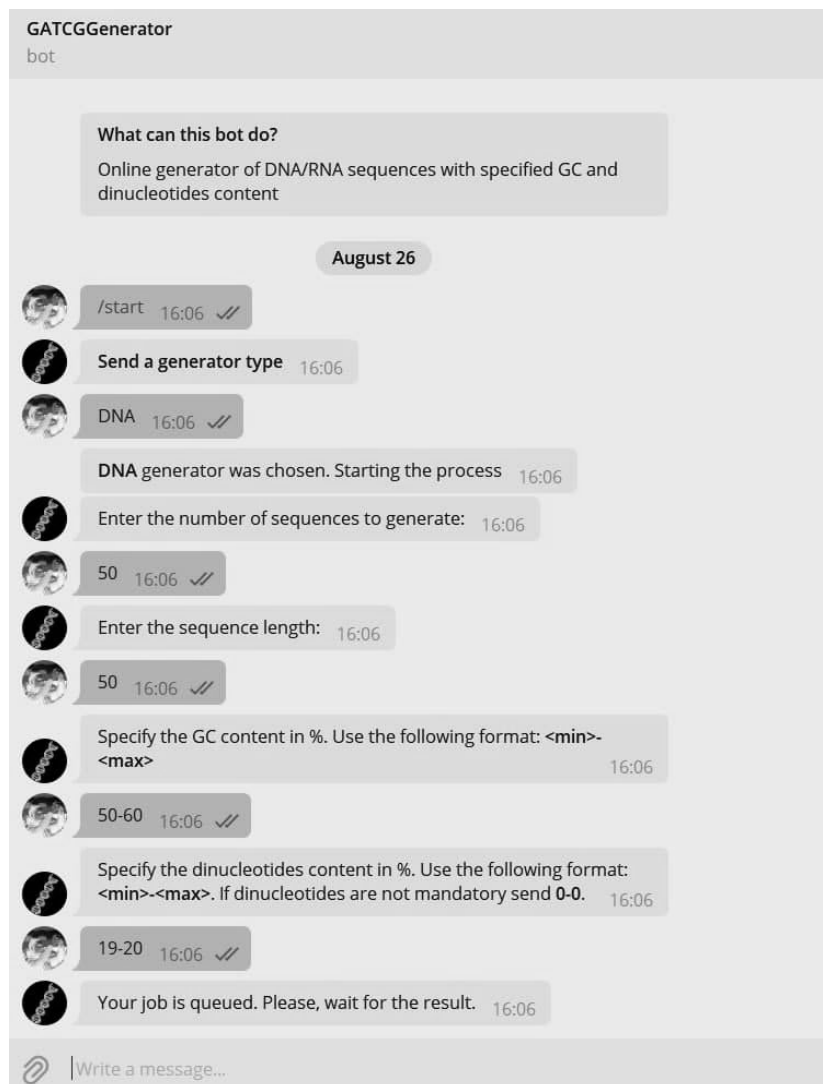


Fig. 1. Example of a user chat in Telegram

In the framework of the presented work, the functionality of random sequence generators and GATCGGenerator were compared. The differences between the input parameters and the specific nucleotide structures obtained as a result of the program were determined (Table 1).

Table 1

Comparison of GATCGGenerator functionality to other nucleotide sequence generators

	GATCGGenerator [20]	Nucleotide Sequence Generator <sup>8</sup>	DNA Sequence Tools: Random Sequence Generator <sup>9</sup>	Random DNA Sequence Generator <sup>10</sup>	Random DNA Sequence <sup>11</sup>	Random DNA Generator <sup>12</sup>
Maximum length (nucleotides)	5,000	1,000,000			10,000	1,000
Number of sequences	100	1			1; 10; 50; 100	100
Input GC composition (%)	+	−	+		−	+ (*)
GC composition (%)	interval		number		−	number
Input NN composition (%)		−				
No homopolymer sites						
Sequence type	DNA/RNA	DNA	DNA/RNA / Protein	DNA		
Output of results	.CSV file	Text on the screen				
(*) User enters AT composition						

GATCGGenerator has a broader functionality, it allows the user to specify the number of dinucleotides, create sequences without extended homopolymer sites and repeats that affect the success of the experiment. In existing generators, it is only possible to vary the GC composition.

The program created by the authors of this research generates a given number of quasi-random sequences of nucleotides that do not have homology with natural DNA, but are suitable for molecular biological manipulations.

**Research Results.** GATCGGenerator allows you to generate specific DNA or RNA sequences from 20 to 5,000 nucleotides long, containing a given number of dinucleotides and not containing homopolymer sites (no more than two identical nucleotides located side by side). More stringent generation conditions can cause a long selection of sequences. As an example, we give a small range of possible content of guanine and cytosine and dinucleotides (e.g., GC composition 45–50 % and NN composition 10–20 %). The operating period of the program for various input data is shown in Table 2.

Table 2

Sequence generation time for different inputs

Data inputs				Time (s)
Length	Number	GC, %	NN, %	
20	10	50–60	20–50	3.45
30	10	50–60	20–50	3.91
20	10	50–60	40–50	9.74
30	10	50–60	40–50	9.53
30	10	40–50	20–20	8.80
1,000	100	45–50	40–50	11.49
2,000	100	45–50	10–20	240.25
5,000	100	50–60	20–50	11.57



GATCGGenerator, through more stringent sequence generation conditions, removes the limitations of known DNA generators and creates quasi-random sequences of nucleotides depending on the indicated input parameters. You can specify the required number of sequences, their length, GC composition and dinucleotide content, as well as the nature of the nucleic acid (DNA or RNA). Specifically, sequences created by GATCGGenerator can be used in DNA steganography, applied to protect and transmit information through hiding the message content in the nucleotide sequence [3].

The proposed software solution (GATCGGenerator) provides obtaining a set of quasi-random sequences of nucleotides depending on user-defined input parameters (type of nucleic acid, sequence length, GC and dinucleotide composition). GATCGGenerator excludes the presence of any nucleotide repeats and homopolymer sites longer than three elements. The generated sequences can be used as service or masking sequences (e.g., in DNA steganography) and are suitable for any non-biological enzymatic manipulations. It is possible to generate numerous artificial nucleotide sequences and use them to create a universal oligotheca suitable for multiple encoding of non-biological data and their long-term storage.

The data presented in Table 3 summarizes the results of the program. For a certain type of nucleic acid (in this case, DNA), the following data is shown: the content of dinucleotides (NN %), the number of generated sequences, their length (nucleotides — nt), and GC composition.

Table 3

Examples of short sequences differing in length, GC composition, and dinucleotide content (%)

Input parameters					Nucleotide sequence, 5'→3'	Output parameters		
Type	Number	Length, nt	GC, %	NN, %*		Length, nt	GC, %	NN, %*
DNA	5	30	41–50	20	CT <b>GG</b> **TATATC <b>GG</b> AATCATATCGCGCAGTGT	30	46.7	20.0
					AATCAGCTAGTAG <b>G</b> ACGCAGTAGTGA <b>AT</b> CA	30	43.3	20.0
					GAATGTAGT <b>C</b> CTAG <b>G</b> CACATACTACGTAGC	30	46.7	20.0
					AG <b>TT</b> GCACTGAAGTCTATGATCT <b>GG</b> CATGC	30	46.7	20.0
					GACACACTACTAT <b>GG</b> ACGTG <b>AGG</b> CACT <b>T</b> AC	30	50.0	20.0
	5		51–60		TCAGCTCAGCG <b>CC</b> AATCGAGCT <b>T</b> TATAGTGC	30	53.3	20.0
					G <b>AGG</b> CTATCGT <b>CA</b> AGCATAG <b>AC</b> CGTGTGCT	30	53.3	20.0
					GACTCAGTAGCTGCT <b>CCG</b> GACATACAGCCT	30	56.7	20.0
					TCGCGCG <b>T</b> TAGACT <b>T</b> AG <b>GT</b> TCTCATCGCAGC	30	56.7	20.0
					ACGCTCAC <b>AGG</b> AG <b>TT</b> CGCATCG <b>AA</b> CGATGC	30	56.7	20.0
	5	41–50	0	ACGACAGTGATATAGCACGACGTGCTCATA	30	46.7	0.0	
				GACTACATCTGATAGTACACGTGCTGCACT	30	46.7	0.0	
				TCTATCTCTGCTAGAGCGCTCGTCACTCTA	30	50.0	0.0	
				TCTGATCTACTATAGCGATACGTGAGAGTG	30	43.3	0.0	
				ACACATATATCGACGCACGCGTCGTAGTAC	30	50.0	0.0	
	5	50	41–60	20	TGCATGACCATGCTTG <b>C</b> GGTAGAC <b>ATT</b> CA GACGCGC <b>GA</b> ATAGTAG <b>G</b> ACGA	50	52.0	20.0
					GCATACGAGT <b>G</b> GCATACAT <b>ATT</b> AGACTAT AC <b>GG</b> TAGTGATAT <b>G</b> GTG <b>CA</b> A	50	42.0	20.0
					CTGAGACT <b>C</b> CTCTCTGT <b>G</b> GAGCT <b>C</b> CTAGTA <b>CC</b> GT <b>C</b> ACGCGTGCTCT <b>GA</b> AG	50	58.0	20.0
					CTGTGT <b>GA</b> ACATACGATGC <b>ATT</b> CTCATCTC <b>GG</b> TAT <b>GG</b> CT <b>GA</b> AGTGCACAT	50	46.0	20.0
					GCGCTGACGTCAT <b>G</b> GTTCATAC <b>CA</b> ATGTA GCATGATGTGCGAT <b>AGG</b> CACA	50	50.0	20.0

\* NN shows the fraction (%) of the dinucleotides contained in the nucleotide sequence.

\*\* Dinucleotides are highlighted in bold.

\* NN shows the fraction (%) of the dinucleotides contained in the nucleotide sequence.

\*\* Dinucleotides are highlighted in bold.

The obtained 30-nucleotide sequences were tested using the Blast tool from NCBI. The absence of 100% homology with known DNA sequences of living organisms was identified. The maximum coincidence was observed for the generated sequences with a length of 25 nucleotides (similarity is about 80 %). This indicates the ability of the GATCGGenerator to generate non-biological nucleotide sequences with high efficiency. It can be assumed that the sequences generated in this way do not have an absolute coincidence with the nucleotide fragments of living organisms.

In this case, special DNA-oligonucleotides of artificial origin containing informative and service parts can be used as a convenient information carrier. Recently, the authors of this work have proposed the use of NYRN-oligonucleotides [14] consisting of:

- internal part (YR)<sub>n</sub> encoding the encrypted information;
- service (auxiliary) parts S1 and S2 flanking sequence (YR)<sub>n</sub> (Fig. 2).

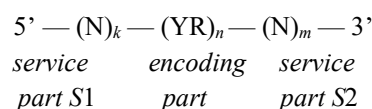


Fig. 2. Structure of NYRN-oligonucleotides: N — degenerate nucleotides; Y — pyrimidines (C or T); R — purines (A or G); *k, n, m* — indices corresponding to the length of the part

The length of the sites (*n*, *k* and *m*) may vary, but the structure of the service parts should provide the successful course of amplification reactions (length more than 18 nt, 40–60 % GC composition, absence of homopolymer sites and repeats). GATCGGenerator allows including NN dinucleotides containing identical paired nucleotides (e.g., AA, GG, CC, TT or UU for RNA), which can increase the specificity of molecular hybridization of nucleic acids.

**Discussion and Conclusion.** Thus, based on the results of the scientific investigation performed, a software solution (GATCGGenerator) has been proposed, which, in comparison to traditional approaches, assumes more stringent conditions for generating sequences. Due to this feature, the limitations of known DNA generators are removed, and quasi-random sequences of nucleotides are formed depending on the specified input parameters. The obtained 30-nucleotide sequences were studied. The test allowed us to establish the absence of 100 % homology with known DNA sequences of living organisms. The generated sequences with a length of 25 nucleotides coincided as much as possible (by about 80 %).

Note also that in order to hide information in NYRN-oligonucleotides, it is required to mix them with masking DNA. Masking sequences should be similar to sequences of NYRN-oligonucleotides, so that when trying to read hidden information, it would be impossible to recognize them without key sequences. The addressee should know the key sequences — primers to the service sites of NYRN-oligonucleotides. The addressee can decipher the transmitted message by isolating informative nucleotide sequences using a polymerase chain reaction followed by sequencing. A set of NYRN- and masking oligonucleotides can be easily obtained using GATCGGenerator, synthesized, and then stored as an oligotheca. To do this, it is enough to determine the optimal NYRN-oligonucleotides with subsequent filling of the oligotheca. In the future, it is planned to conduct laboratory experiments in order to test the proposed method of storing non-biological information and checking the viability of oligotheca obtained using the generator.

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